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Quarterly Progress to: National Institutes of Health

Contract Monitor William Heetderks, Ph.D.

Research Contract: Surface Modification for Biocompatibility

Contract Number: NS 5-2322

Principal Investigators: David C. Martin and K. Sue O'Shea

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The Martin Laboratory:

Polymer Production:

As reported earlier, Mike Johnson, a graduate student in the lab, spent several weeks at Protein Polymer Technologies Inc., in San Diego, to synthesize additional SLPL. The new SLPL (batch 8) has been re-purified, and is being tested. The protocol involved extensive dialysis after dissolution in formic acid. The sample was tested at Desert Analytics, Tucson, AZ for chemical composition, and a western blot performed at PPTI, San Diego, CA for molecular weight analysis. The chemical analysis showed the resulting material to be 90% pure, with residual formate salt. The western blot indicated that the material had not degraded within the limits of the test. The remainder of the material will be re-purified with attention to removing the residual salts. The material will also be analyzed for chemistry and molecular weight.

Probe Coating:

Investigations are underway to examine possible ways to create a more consistent gradient in fiber density in order to enhance the mechanical matching between the hard probe and the soft tissue. We hope to take advantage of at least two morphologies available from the electrospinning process. At short distances (less than 2 cm), fibers are deposited while still partially solvated which encourages adhesion to the surface and to other fibers. As the distance is increased between the spinneret and the substrate, the solvent evaporates and the fibers splay. The fibers go down in a less dense pattern, with fibers curving both on the substrate as well as out of the plane. The two morphologies will be deposited sequentially to decrease the density from probe to tissue using two methods; by movement of the substrate during the deposition process, and by deposition in two separate steps. The resulting coatings will be examined by SEM.

FEM Simulation of *B. mori* Silk Fibers

Bombyx mori cocoon silk was sectioned axially and examined using TEM. Roughly parabolic bands were found with a periodicity of approximately 600 nm. Finite element models were created to examine the effects of this morphology on the mechanical behavior (Figure 1). The contrast was attributed to mass-thickness differences after the de-gumming process. It was assumed that the darker areas were the crystalline phase and the lower electron dense areas were mostly amorphous. The modulus was estimated to be 100 times greater in the silk II crystal than in the amorphous phase. The difference in dimension was approximately 4 to 1 for the RhardS to the RsoftS phase. Therefore, three control and two test models were created using the parameters for dimension, modulus and yield point. For the control models, bands of hard and soft material were layered at 0, 45, and 90 degrees to the axis. The two test models were parabolic and v-shaped layers of the phases. The fiber dimension, band spacing, modulus, yield, and amount of deformation were the same for all simulations.

In the control models, the modulus, yield and necking was consistent with experimental load-displacement curves. In the test models, however, the onset of necking did not occur until a much greater strain (Figure 2). Examination of the element map showed a redistribution of the stresses from the center to the fiber surface through shear deformation of the soft phase (Figure 3). The mechanical modification due to the geometry of the banded hard and soft phases is significant and is a process parameter independent of the chemistry and mechanical properties of the individual phases.

FEM Simulation of Probe in Brain

A model of a Si probe embedded in the brain was built using finite elements programs (Figure 4). The probe, brain, cranium, meninges and agar were incorporated into the model. The mechanical properties were taken from a variety of sources or estimated from other tissues such as collagen and elastin. Simulations of movement of the brain, cranium and probe are under way in order to examine stresses in the system and to highlight which areas would benefit from the softer interface provided by the coating between brain and probe.

O'Shea lab

Work is actively ongoing in analyzing cell migration, cell and process morphology on the various coated substrates. The analysis is being carried out by Dr. Libby Louie, who is working to prepare the paper for publication. There continues to be tremendous interest in using the patterned substrates for studies of neuronal substrate preference, studies of macrophage behavior, basic cell biological work, and we are currently investigating funding mechanisms to allow us to produce the substrates in Ann Arbor, rather than at the Cornell Nanofabrication laboratory.

This winter we submitted an equipment grant to the NIH to partially cover the cost of purchasing a new confocal microscope for the Cell Biology Laboratories in the department of Anatomy and Cell Biology, here at the University of Michigan. If funded, this equipment would significantly expedite the probe - tissue interface analysis. Chris Edwards, CBL manager and Pete Finger in the Altschuler lab were able to obtain a reflection image of the probe in the Guinea pig brain (Figure 5) using the Zeiss equipment. We are all very excited by the opportunity to embed using LR White resin, which will allow antibody localization, in combination with the reflection mode, which will allow precise localization of the electrode shaft and tip. Since we have antibodies to the SLP, we will be in position to examine cell-coating as well as cell-electrode interactions!

Collaboration with the Mensinger Lab

Si microprobes were coated with SLPL by the electrospinning process. Consistent with previous runs, the tip was coated while the shank and the terminal pad were covered with a glass coverslip. Both sides of the tip were coated with SLPL and delivered to the Mensinger group at Washington University.

Allen reports that "40-60% of the pores of the coated probes have neural tissue growing through the pores while the uncoated probes had less than 5% of the pores filled". They are now analyzing pore size to determine which diameter holes are preferred by neurites. Very promising results!

Fig. [illegible] 1

FINITE ELMENT MODELS

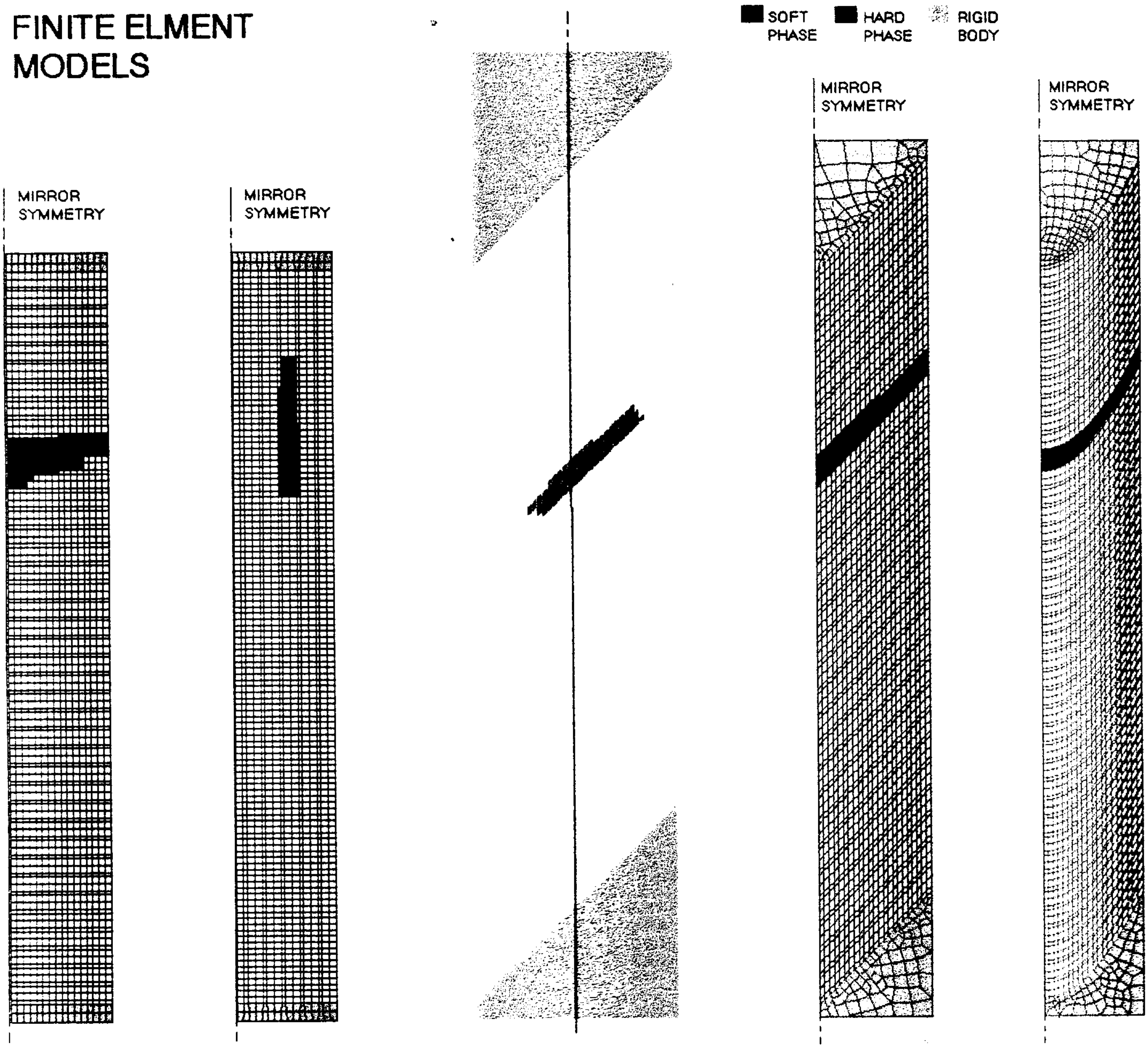
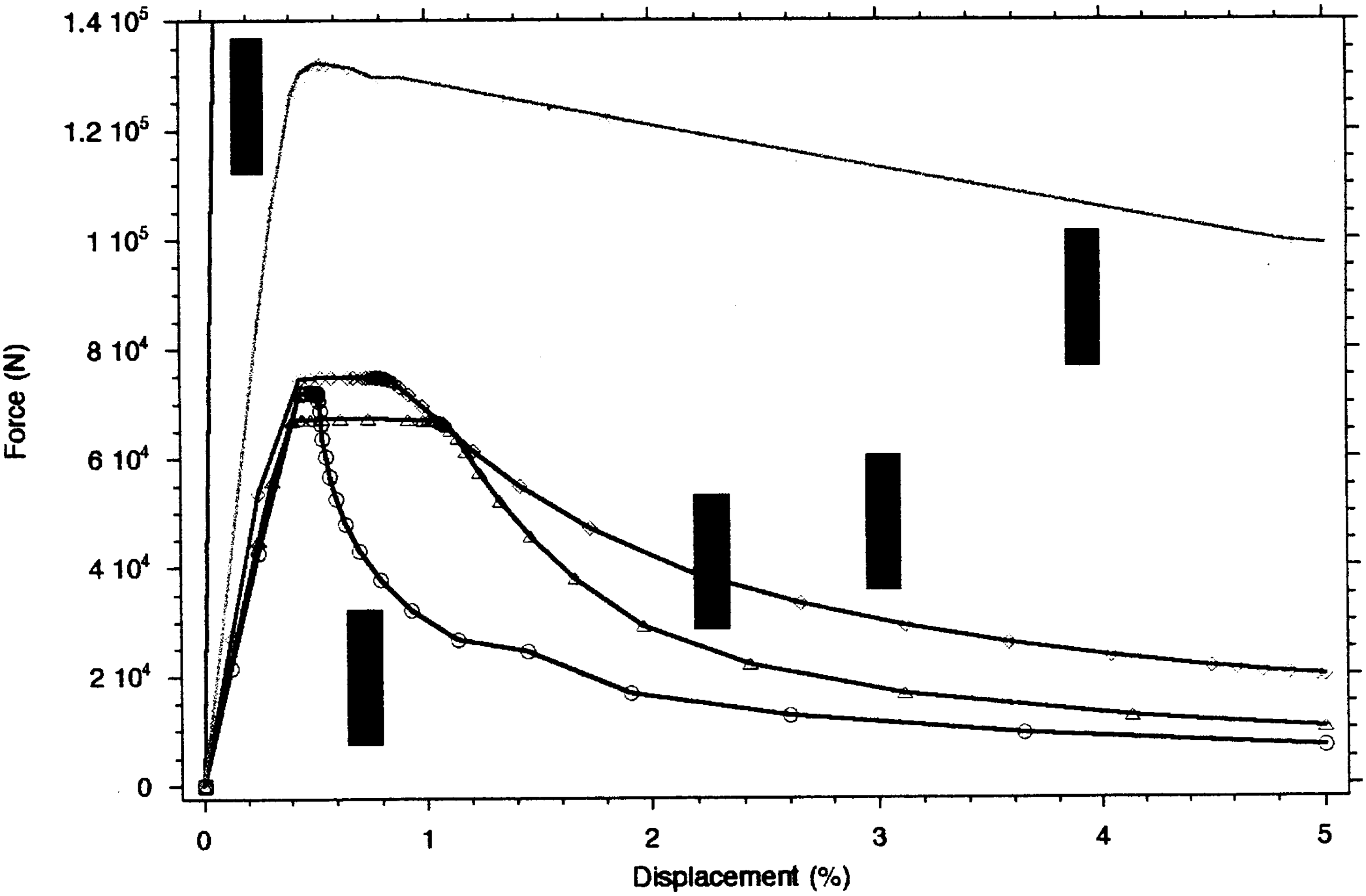
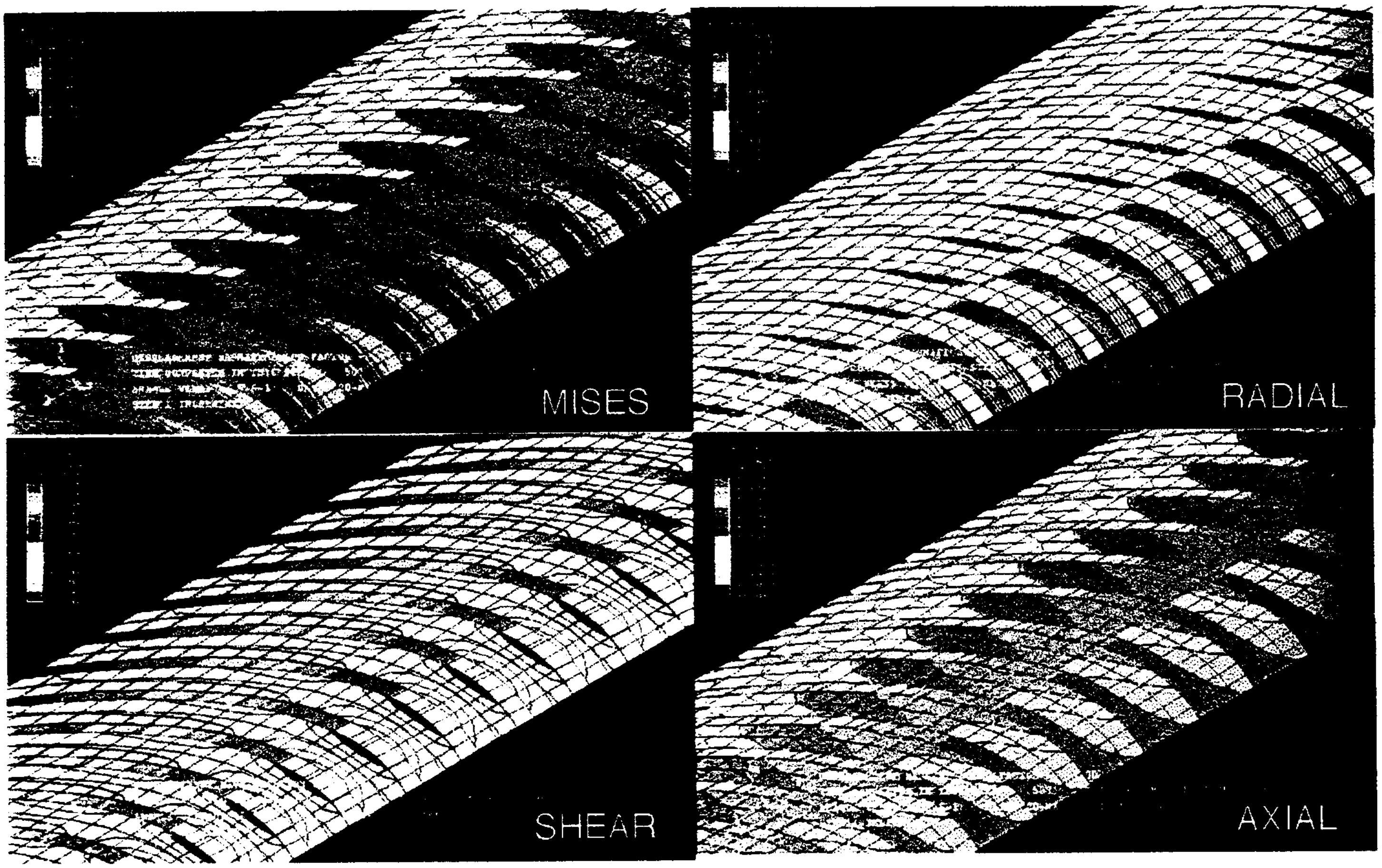


Figure 2.

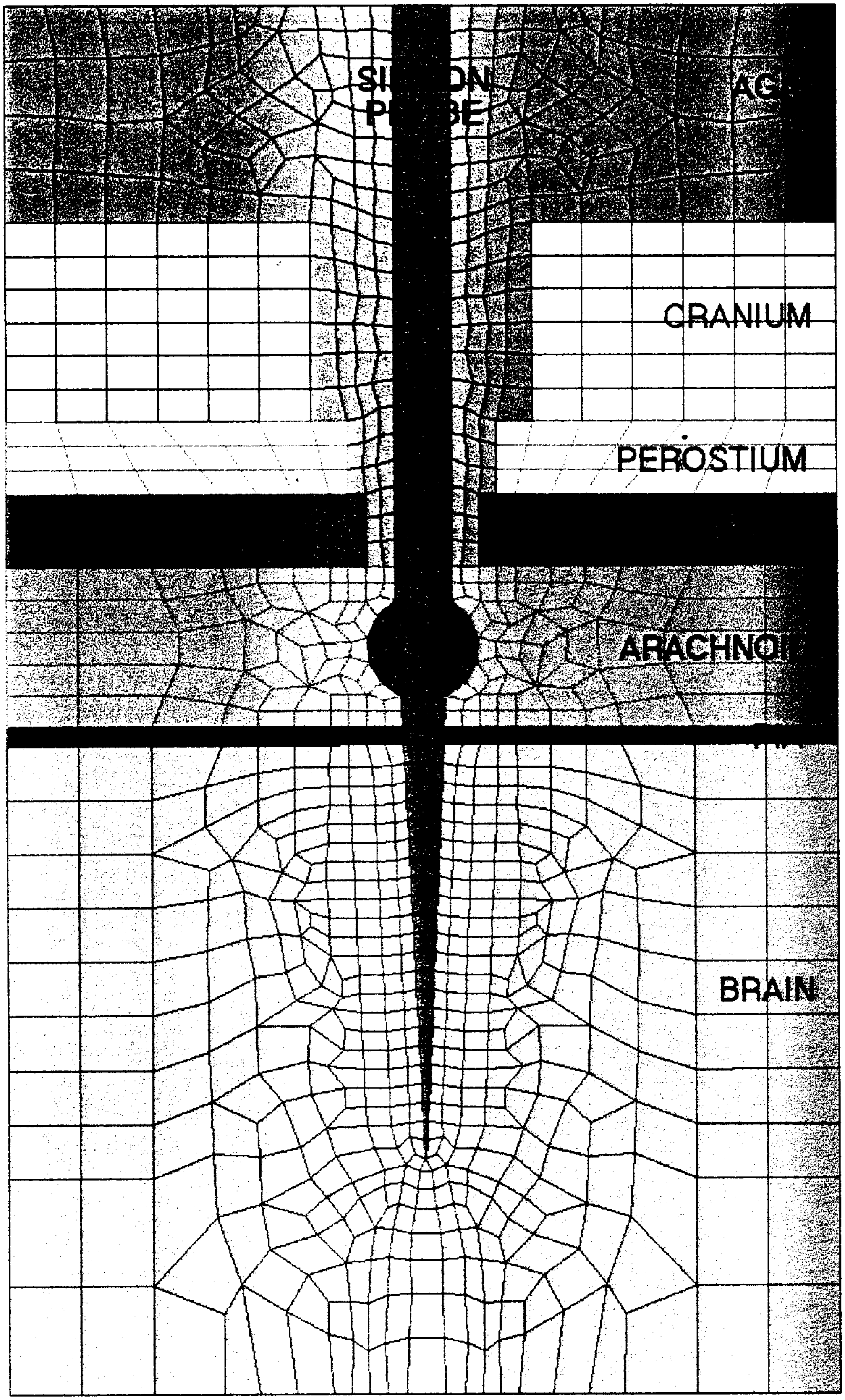
Comparison of FE Models, elastic/plastic@2%, 100:1 modulus ratio





Stress fields for the parabolic geometry of a banded fiber at 5% elongation

Figure 4



Finite element model of Si probe in the brain

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